REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	2004	Journal Article-Journal of Thermal Biology

4. TITLE AND SUBTITLE
Heart Rate Variability as an Index of Physiological Strain in Hyperthermic and Dehydrated Rats

5. FUNDING NUMBERS

6. AUTHOR(S)
C.B. Matthew, A.M. Bastille, R.R. Gonzalez, I.V. Sils, .W. Hoyt

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Thermal & Mountain Medicine Division
U.S. Army Research Institute of Environmental Medicine
Kansas Street
Natick, MA 01760-5007

8. PERFORMING ORGANIZATION REPORT NUMBER
M03-44

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Same as #7 above

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
Telemetry-equipped rats were subjected to hyperthertnia (H, Tc = 41.5°C) with and without dehydration (D, 9% loss of body weight) stresses. Time domain and spectral analysis of heart rate variability indicated that the dehydrated hyperthermic (DH) rats had increased sympathetic activity compared to H rats, and DH rats had decreased parasympathetic activity compared to less hyperthermic D rats. These non-invasive measures of physiological strain discriminated the more physiologically stressful of these states.

14. SUBJECT TERMS
hyperthermia; dehydration; heart rate variability; central control of cardiac frequency; autonomic nervous system

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unclassified

ELSEVIER

Journal of Thermal Biology 29 (2004) 211–219

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Heart rate variability as an index of physiological strain in hyperthermic and dehydrated rats

Candace B. Matthew[a,*], Amy M. Bastille[a], Richard R. Gonzalez[b], Ingrid V. Sils[a], Reed W. Hoyt[b]

[a] *Thermal and Mountain Medicine Division, USARIEM, Kansas St, Natick, MA 01760-5007, USA*
[b] *Biophysical and Biomedical Modeling Division, USARIEM, Kansas St, Natick, MA 01760-5007, USA*

Abstract

Telemetry-equipped rats were subjected to hyperthermia (H, $T_c = 41.5°C$) with and without dehydration (D, 9% loss of body weight) stresses. Time domain and spectral analysis of heart rate variability indicated that the dehydrated hyperthermic (DH) rats had increased sympathetic activity compared to H rats, and DH rats had decreased parasympathetic activity compared to less hyperthermic D rats. These non-invasive measures of physiological strain discriminated the more physiologically stressful of these states.
Published by Elsevier Ltd.

Keywords: Hyperthermia; Dehydration; Heart rate variability; Central control of cardiac frequency; Autonomic nervous system

1. Introduction

The objective of this study was to evaluate the use of a variety of methods of time domain and spectral analysis of heart rate variability (HRV) as markers of physiological status during hyperthermia with and without dehydration, and dehydration alone in a rat model applicable to human heat strain. The ultimate goal is to identify autonomic nervous system markers derived from measurements made by Warfighter Physiological Status Monitor (WPSM). The WPSM, a wearable device under development by the US Army, will gather information on thermal strain, hydration status, energy balance, sleep status, cognitive function, and vital signs to which algorithms would be applied to determine the individual's status and preparedness. Autonomic nervous system markers serve as a useful means of evaluating current and predicting near-term physiological (i.e. neural control of circulation) status. Such information could enhance survivability by predicting eminent cardiovascular collapse early enough for successful intervention. An animal model is required for this work, as it requires examination of physiological responses at core temperature (T_c) and hydration levels too dangerous for the use of volunteers.

HRV refers to variation among R–R intervals (consecutive QRS complexes) and variation in instantaneous heart rate (HR). Evidence of an association between lethal arrhythmias and an increase in sympathetic and/or decrease in parasympathetic activity has stimulated efforts to identify quantifiable markers of this autonomic nervous system activity (Task Force, 1996). In all, 75–90% of HRV has been attributed to variable fluctuations due to thermoregulatory modulation of peripheral blood flow, fluctuations arising from central nervous system blood pressure (BP) control, and respiration (Sayers, 1973) with the remainder due to non-neuronal factors, such as atrial stretch and changes in intrathoracic pressure (Casadei et al., 1995). Since these variations occur at different frequencies, frequency selective analysis of interbeat variation may allow separation of the contributions of each of these components.

Sinus arrhythmia is the irregular heart rhythm related to the phases of respiration that increases during inspiration and decreases during expiration. The central

*Corresponding author. Tel.: +1-508-435-3265; fax: +1-508-233-5298.
E-mail address: candbio@aol.com (C.B. Matthew).

0306-4565/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.jtherbio.2004.03.001

frequency of the high frequency (HF) peak of the HRV spectrum is identical to the peak generated by measuring respiration alone thus verifying that respiratory rhythm is directly associated with the HF peak (Perlini et al., 1995). The low frequency (LF) peak is associated with vasomotor tone linked to BP maintenance as demonstrated by the change in this portion of this peak associated with the BP changes seen on tilting or standing in humans (Lipsitz et al., 1990). Similarly, the very low frequency (VLF) band of the HRV spectrum may be entrained by immersing the hand of subjects alternately in hot and cool water (Kitney, 1975) thus confirming that this is a thermoregulatory frequency band.

The time domain analyses used in this study were interbeat interval (IBI) and normalized standard deviation (NSD = standard deviation/mean IBI). Frequency domain analysis or spectral analysis generates a cumulative power spectrum of a series of IBIs. Power is defined as the square of variance, and total power is estimated by the standard deviation of all IBIs (Kleiger et al., 1995). In rats, the definitions of HF (0.75–3 Hz), mid-frequency (MF, equivalent to LF in humans, 0.25–0.75 Hz) and LF (equivalent to VLF in humans, 0.02–0.25 Hz) differ from that of humans only in frequency bands due to the higher heart and respiratory rates of rats. Very similar results were obtained in analogous bands on intravenous administration of atropine (parasympathetic blocker) and propranolol (sympathetic blocker) to rats (Cerutti et al., 1991; Perlini et al., 1995) as in humans (Pomeranz et al., 1985). Also as with humans, HRV in rats is due to autonomic and non-autonomic etiologies. Efferent vagal input controls the major portion of the sinus arrhythmia, but other factors such as the mechanical effects of ventilation are also involved (Casadei et al., 1995).

In this study, HRV was analyzed in rats exposed to hyperthermia with and without dehydration, and dehydration alone to determine autonomic nervous system signatures that might be used to differentiate among these physiological conditions. Such HRV analysis could potentially have use in the development of algorithms for use in physiological monitoring.

2. Methods

2.1. Animals

All experimental procedures were approved by our Institutional Animal Care and Use Committee and carried out with adherence to the "Guide for the Care and Use of Laboratory Animals", as revised in 1996 and to the US Government Principles for Animal Use, 1985. Animals were cared for and observed by our staff of credentialed animal care technicians headed by a staff veterinarian.

Three groups of eight rats each (*Rattus norvegicus*, male, Harlan Sprague–Dawley, 428 ± 28 g) were used as follows: desalivated restrained (DesalR) and desalivated unrestrained (DesalUR) were the two hyperthermic (H) groups, dehydrated hyperthermic (DH), and dehydrated (D). The DesalR and DesalUR rats were the same rats; with each rat acting as its own control for restraint effects. The DesalR rats were heat stressed while restrained to yield a hyperthermic group with minimal loss of body water; the DesalUR rats were heat stressed while unrestrained to determine which if any results seen in the first group were due to restraint; the DH rats were not surgically desalivated but were heat stressed unrestrained to yield a group that was both hyperthermic and dehydrated; and the D group was dehydrated without attaining as great a level of hyperthermia. For the first heat exposure, half of the Desal rats were heat exposed while restrained and the remaining half were heat exposed while unrestrained; on the second exposures the restraint was reversed to control for possible repeated exposure effects with at least 1 week between exposures. All animals were housed in individual wire-bottomed cages in the animal colony until the start of the experiment. Environmental conditions were maintained ($T_a = 26^\circ$C, 50% rh), lighting was controlled automatically (on, 0600–1800 h), and food (Harlan, Teklad LM-485) and water were available ad libitum except during experimental intervals.

2.2. Surgical preparation

Since rats spread saliva for evaporative cooling (analogous to evaporation of sweat in humans) one group of eight rats was surgically desalivated (ligation and transection of the parotid, submaxillary and major sublingual glands to achieve hyperthermia without dehydration. Desalivation was done under pentobarbital anesthesia (Nembutal, 45 mg/kg, intraperitoneal; atropine, 200 μg, intramuscularly; Polyflex® ampicillin, 12.5 mg, intramuscularly) using aseptic technique. Desalivation was done 2 weeks prior to telemetry implantation to allow time for the rats to learn to eat with minimal saliva (increased drinking and food wastage) and to regain pre-surgical weight levels.

All animals had a telemetry device (Data Sciences, St. Paul, MN, USA, TL11M2-C50-PXT, core temperature (T_c), BP, HR, and ECG waveforms) surgically implanted, 2 weeks prior to experimentation as previously described (Matthew, 1997) under the same anesthetic regimen as for the desalivation outlined above.

2.3. Experimental procedure

Baseline data were collected on a day prior to heat stress while the animals were unrestrained. DesalR, DesalUR, and DH groups were exposed to a

$T_a = 41.5°C$ until a T_c of 41.5°C was attained. In order to have a group with minimal water loss (weight loss) the DesalR group was subjected to heat stress in a restraining cage as previously described (Hubbard et al., 1982; Matthew et al., 1986). Desal rats were placed in restraining cages 3× for 45 min each time the week prior to the heat exposure to acclimate the animals to the restraint cages and thus minimize any impact of restraint stress. The other three groups were heat stressed while unrestrained in their own home cages. D rats were exposed to a T_a of 38°C until they achieved the same level of dehydration (weight loss) as the DH group. Rats were placed in an insulted container and quickly removed from the heat once each hour for body weight determination; previous work (Hubbard et al., 1977, 1982; Matthew et al., 1986) has shown that the rate of water loss is fairly linear in unrestrained heat exposed rats. On reaching a T_c of 41.5°C for the H groups and an ~9% dehydration for the D group, the rats were removed from the heat chamber and allowed to cool passively.

2.4. Data analysis

Since the normothermic rat has an HR in excess of 300 bpm, 1 min of continuous data provided sufficient data points for PSD analysis. IBIs and NSD were determined and then PSD was performed on the IBI data as follows: IBIs were extracted from the ECG waveforms using a computer program developed in our laboratory; power spectra of IBI data series were produced using Welsh's method (Marple, 1987). Unit time differenced data were used, and spectral decomposition was done using the PSD function of MatLab® software with overlapping Hanning windows. PSD was calculated at baseline (collected on a day prior to the heat stress) and during heating at each half a degree increase in T_c (39°C, 39.5°C, 40°C, 40.5°C, 41°C, 41.5°C (end of heat, EOH) T_c max, and each half a degree during cooling. Areas under the PSD profile were divided into LF (0.02–0.25 Hz), MF (0.25–0.75 Hz), and HF (0.75–3.0 Hz). Hyperthermic area (HA) in deg min above a T_c of 40.4°C was also calculated for each animal as follows: HA = ∑interval length (min) × $\frac{1}{2}$ (°C above 40.4 start of interval + °C above 40.4 end of interval) (Hubbard et al., 1977). All values are reported as mean ± SD. Significance testing was determined by MANOVA followed by Tukey's post hoc testing; significance was accepted at the $p < 0.05$ level.

3. Results

Table 1 contains the heat exposure time required, % weight loss (as an index of water loss dehydration) incurred, the thermal area accumulated, and the mean maximum T_c for DesalR, DesalUR, and DH groups to reach a T_c of 41.5°C and for the D group to attain ~9% loss of body weight. The end-point chosen for the D group was ~9% loss of body weight seen in the DH groups on reaching a T_c end-point of 41.5°C. The DesalR group was the fastest to achieve the hyperthermic end-point with the least amount of dehydration. DH rats, that were able to freely spread saliva for evaporative cooling, maintained a thermoregulatory plateau for an extended period of time requiring 290 ± 71 vs. 35 ± 5 min in the DesalR group to attain a T_c of 41.5°C. All of the DH rats reached a T_c of 41.5°C; only one D rat reached a T_c of 41.0°C while most remained below 40.4°C. No statistical significance testing was done on the data in Table 1, because these variables were artificially controlled to achieve the desired end points.

Figs. 1 and 2 present data comparing the three hyperthermic groups (DesalR, DesalUR, DH) at baseline, at each 0.5°C increase in T_c during heating and at each 0.5°C decrease during cooling. The time that the animals reach 41.5°C was defined as EOH, and as the T_c frequently continued to increase after removal from the heat (0.03–0.4°C) to a maximum (Max T_c) prior to cooling. There was a significant decrease in IBI (Fig. 1a) through heating and an increase through cooling. Across the whole heat stress interval the DH group had a significantly higher IBI; there were no consistent significant differences in IBI among the three groups during cooling. As expected, HR data collected during the acclimation to restraint sessions indicated that the R rats had a significantly elevated HR compared to the UR rats (385 ± 34 vs. 341 ± 31, $p < 0.001$). However, during hyperthermia induction there was no significant

Table 1
Heat time, weight loss, and thermal area for all groups

Group	Heat time (min)	%wt loss (%)	Total area (deg min)	Max T_c (°C)
DesalR	35 ± 5	1.0 ± 0.4	23 ± 3	41.9 ± 0.1
DesalUR	49 ± 11	1.8 ± 0.4	25 ± 3	41.7 ± 0.1
Dehyd. heat	290 ± 71	8.7 ± 1.6	53 ± 12	41.6 ± 0.1
Dehyd.	320 ± 68	8.9 ± 0.3	12 ± 16	40.4 ± 0.4

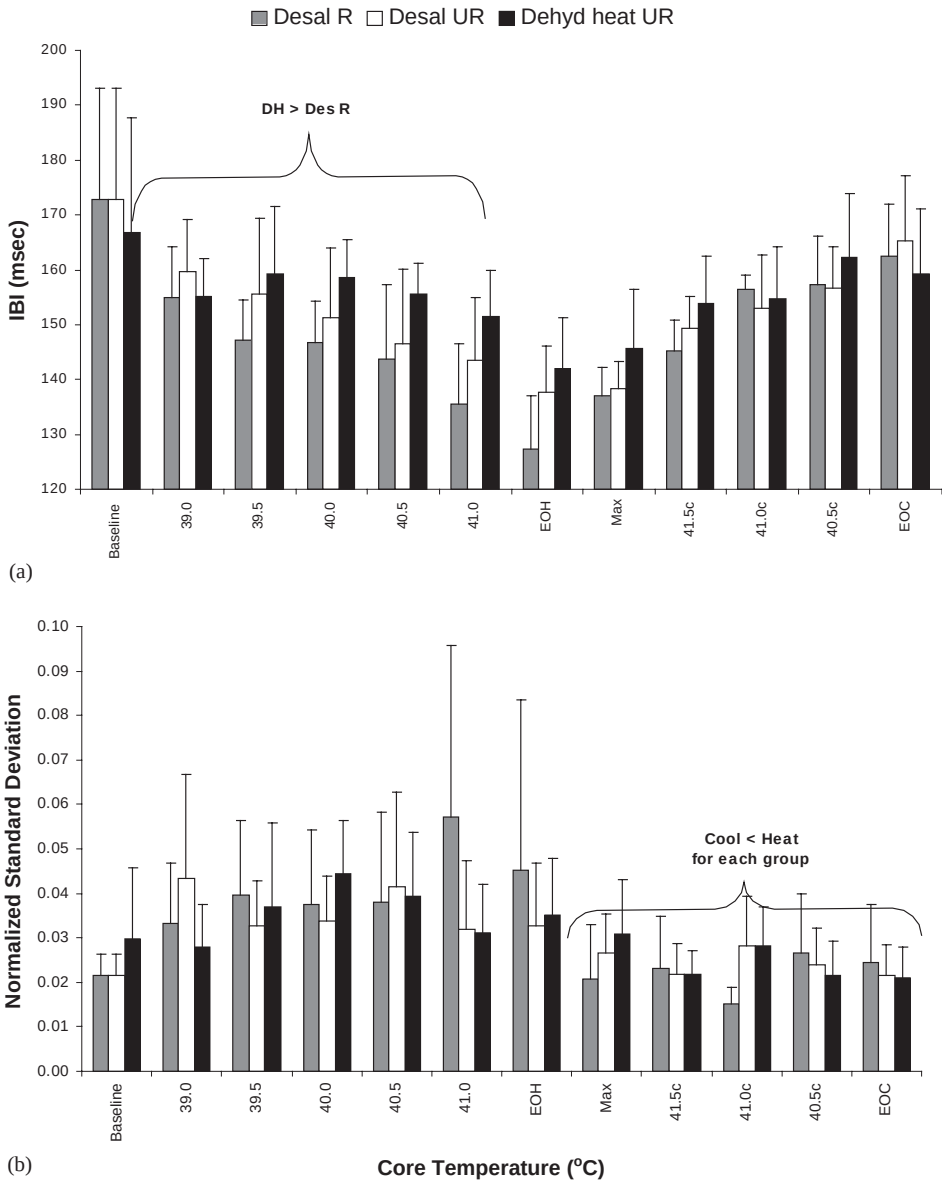

Fig. 1. (a) IBI for the three groups subjected to a heat stress until they attained a T_c of 41.5°C is represented at each half a degree increase in T_c during heating and each half a degree decrease during cooling. The DH group had a significantly ($p<0.05$) greater IBI through heating than the other two groups. (b) NSD for the three groups subjected to a heat stress until they attained a T_c of 41.5°C. The NSD was significantly ($p<0.05$) lower during cooling than during heating for all groups.

difference between R and UR rats (Fig. 1a). In all groups, NSD (Fig. 1b) increased during heating and decreased during cooling. The NSDs were significantly lower (lower total variability) for each group during cooling than during heating.

Fig. 2a contains the area under the PSD in the LF band (0.02–0.25 Hz). Across heating there was a non-significant trend toward a decrease in LF area. The DH group had a significantly greater LF area during cooling than the other groups during cooling and than the DH during heating. Similarly, there was a non-significant trend to decreased MF (0.25–0.75 Hz, Fig. 2b) area through heating. The DH group had a significantly greater MF area during cooling than the other groups during cooling and than the DH during heating. There were no consistent changes in HF (0.75–3.0 Hz, Fig. 2c)

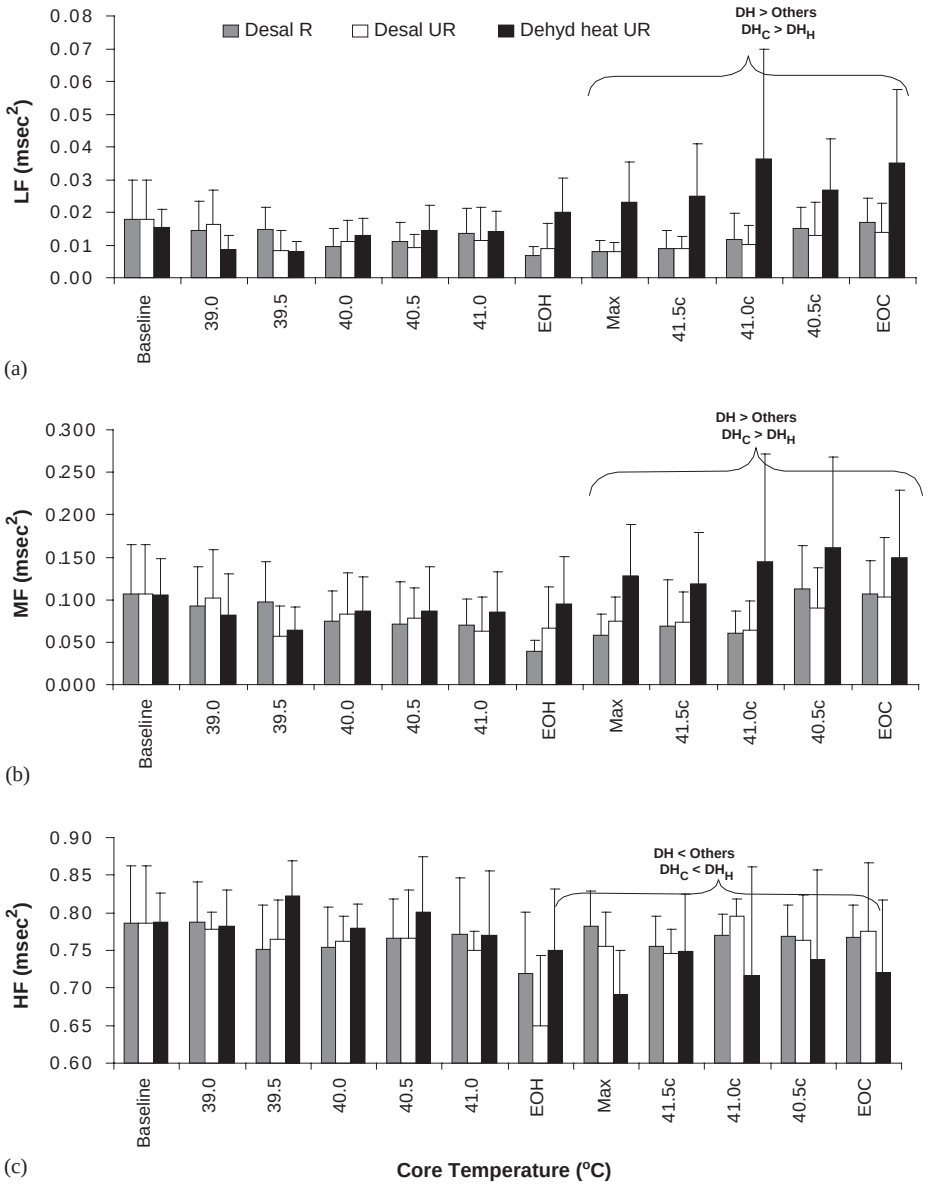

Fig. 2. (a) LF area (ms^2) under the PSD curve for the three groups subjected to a heat stress until they attained a T_c of 41.5°C. The DH group had a significantly ($p<0.05$) higher LF area during cooling (C) than the other groups during cooling and than the DH during heating (H). (b) MF area under the PSD curve. The DH group had a significantly ($p<0.05$) higher MF area during cooling than the other groups during cooling and than the DH during heating. (c) HF area under the PSD curve. The DH group had a significantly ($p<0.05$) lower HF area during cooling than the other groups during cooling and than the DH during heating.

area through heating or cooling for DesalR or DesalUR. However, in contrast to the LF and MF areas, the DH group had a significantly lower HF area during cooling than the other groups during cooling and than the DH during heating.

Figs. 3a and b present data comparing the two dehydrated groups (DH, D) at each 1% decrease in body weight during heating. "0" represents the first minute that the animals were placed in the heated chamber; "00" represents 5 min later when they had settled down and was used as the starting value. There is no value for the DH group at 8% because one or more of the animals reached a T_c of 41.5°C prior to attaining an 8% decrease in body weight. These figures present data collected during heating only. There were no consistent significant differences between D and DH in

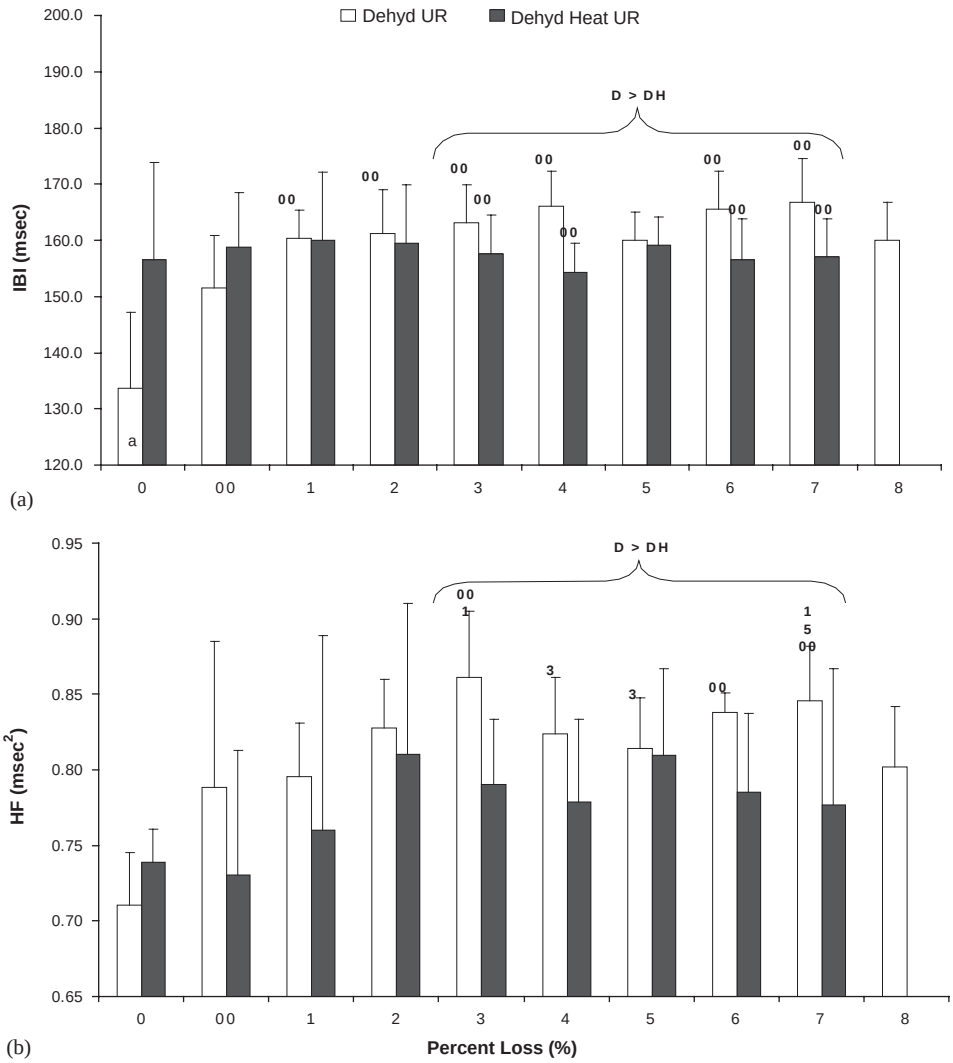

Fig. 3. (a) IBI for the two dehydrated groups is represented at each 1% decrease in body weight during heating. 0 represents the first minute that the animals were placed in the heated chamber; 00 represents 5 min later when they were settled down and is used as the starting value. There is no value for the DH group at 8%, because one or more of the animals reached a T_c of 41.5°C prior to attaining an 8% decrease in body weight. The D group had a significantly higher IBI than the DH group from 3% loss in body weight on. (b) HF area under the PSD curve. The D group had a significantly higher HF than the DH group from 3% loss in body weight on. The symbols 00, 1, 3, 5 identify values that were significantly different ($p<0.05$) from the starting value (00) or the values at 1%, 3%, or 5% dehydration.

NSD, LF, or MF. Fig. 3a depicts the IBI, and Fig. 3b depicts the HF areas for the two dehydrated groups; for both variables the D group was significantly greater than the DH group from 3% dehydration on up. Therefore, the DH group had a higher HR and lower parasympathetic drive than the D group.

Combining the effects noted in Figs. 1–3: changes in T_c resulted in bi-phasic changes in IBI, NSD, LF, and MF; IBI, LF, and MF decreased during heating and increased during cooling, while NSD increased on heating and decreased on cooling. Restraint resulted in a small, transient, non-significant decrease in IBI prior to mean T_c reaching 39°C (data not represented in Fig. 1a). Any effect of restraint during hyperthermia induction was masked by hyperthermia. Hyperthermia with dehydration (DH) increased IBI, LF, and MF but decreased HF compared to T_c effects alone. Dehydration alone increased IBI and HF during heating as compared to DH. The combination of hyperthermia and dehydration resulted in increases in LF and MF (index of sympathetic activity) as well as decreases in HF (index of parasympathetic activity).

4. Discussion

In this study, there were differences in HRV between hyperthermic rats with minimal water loss, rats with dehydration and minimal hyperthermia and rats with both hyperthermia and dehydration. Rats subjected to hot environments spread saliva for evaporative cooling (Hainsworth, 1967) that has been shown to be quantitatively similar and neurologically controlled in a similar manner to thermal sweating in humans (Matthew et al., 1986). In order to obtain animals that would be hyperthermic with minimal dehydration, it was necessary to prevent salivary water loss. Surgical desalivation of the rat prevents the secretion of saliva for evaporative cooling, but unrestrained desalivated rats have been reported to spread urine for evaporative cooling (Hubbard et al., 1982; Matthew et al., 1986). Therefore, in the present study, to elicit hyperthermia with minimal water loss dehydration the DesalR rats were surgically desalivated and heat stressed in a restraining cage. The shorter heat time and smaller weight loss for the DesalR rats compared to the DesalUR (Table 1) was due to the ability of the DesalUR rats to spread some urine and to use changes in posture to expose the maximum surface area for radiative heat loss. However, no other differences were noted between the R and UR rats during hyperthermia induction; therefore, differences noted in HRV in the Desal R rats may be attributed to hyperthermia rather than restraint.

Time domain and spectral analysis of HRV provides a non-invasive method of assessing the status of the cardiovascular system and the autonomic nervous system. Results using HRV are consistent with earlier work with pharmacological blockade (Casadei et al., 1995; Cerutti et al., 1991; Perlini et al., 1995), but spectral analysis has the added advantage of no possible confusion of the results by neurological compensation for the removal of either the PNS or SNS branches of the autonomic nervous system.

The increase in NSD (Fig. 1b, total power) during heat stress is indicative of increased IBI variability. However, this increase is not reflected in increases in LF, MF, or HF area during heat stress. Since changes in these bands have been associated with changes in autonomic nervous system activity (Cerutti et al., 1991; Nelskla et al., 1999; Perlini et al., 1995), the increases in NSD during heating may be attributed to non-neuronal causes. However, diminished total HRV can result from both autonomic withdrawal and excessively high levels of autonomic input (saturation) (Task Force, 1996). The drop in NSD during cooling on removal from the heat may be due to a change from active to a passive cooling mechanism.

Fig. 2a depicts a decrease in LF band during heat stress that, at least for the DH rats, increased on cooling. Since the VLF in human subjects (LF in rats) has been shown to be entrained by alternately immersing the hand in hot and cold water (Kitney, 1975), a decrease in active tone of the vasodilatation or a sustained reduction in vasoconstriction as might be seen under the hot ambient temperature in this study might be expected to minimize fluctuations of this band. As the increase in skin blood flow in the heat can be attributed to the withdrawal of sympathetic vasoconstrictor tone, the decrease in sympathetic activity indicated by a decrease in LF and MF areas during heating as seen in Figs. 2a and b would be expected. Our finding of a decrease in HF power with hyperthermia in the DH (Fig. 2c) group is consistent with the work of Crandall et al. (2000) who reported a reduced HF power in volunteers with experimentally induced hyperthermia with controlled respiration.

In a comparison of the DH and D groups (Fig. 3), the lack of a difference between the two groups in NSD, LF, or MF, where differences were seen in a comparison of the three hyperthermic groups (Figs. 1 and 2), implies that the dehydration may be a greater physiological strain than hyperthermia alone. Additionally, the greater IBI in the D group compared to the DH group is indicative of a higher HR in the DH perhaps due to a reduced central blood volume secondary to a greater shift in the plasma volume to the periphery. Since a decrease in parasympathetic tone (HF) and/or increase in sympathetic tone (LF) is associated with an increase in lethal arrhythmias (Task Force, 1996), the DH group subjected to both hyperthermia and dehydration would have a higher overall physiological strain than the other groups in this study. Studies of autonomic balance in HR control relative to smoking, age, exercise history, and cardiovascular disease have all indicated that a decrease in HF (parasympathetic control) and/or increase in LF (sympathetic control) indicate an increased risk of cardiovascular sequelae (Evans et al., 2001).

Increasing exercise intensity also elicits a change from predominant PNS to SNS influence. During the initiation of exercise, the increase in HR is due to a decrease in parasympathetic input, but as exercise continues and intensity increases sympathetic influence predominates (Vatner and Pagani, 1976). More recently Pichot et al. (2002) reported the ability to achieve the increase in total HRV, shift of the sympathovagal influence toward a more prominent vagal tone and bradycardia commonly seen in trained athletes by intensive training of sedentary individuals. However, when this was followed by a period of overload training the balance shifted to that of a decreasing vagal and increasing sympathetic tone that did not resolve until in 8–23 days after cessation of the overload work. Thus, once again the simultaneous decrease in parasympathetic tone and increase in sympathetic signaled an increase in physiological strain. Perhaps, a shift from predominance of parasympathetic

tone to one of sympathetic tone ought to be recognized as a universal physiological distress signal.

The study by Pichot et al. (2002) provides a means of measuring training effects on total HRV but requires downloading and analysis of data from a Holter monitor after the collection of the data over may hours. The USARIEM vigilance monitor (Lieberman and Coffey, 2000) currently measures activity patterns, vigilance, and environmental conditions and is being used for field study applications where access to the volunteer soldier for more traditional testing is limited. Both of these approaches are valuable for experimental studies but do not provide real time assessment of the individual's current status. The WPSM, however, measures and transmits in real time, on query, at specific intervals, or also stores for later download: location, body temperature, IBI, and foot-strike measurements (Hoyt et al., 2002). The addition of information on net autonomic nervous system status that could be obtained from HRV as measured via IBI would significantly enhance the information available on the physiological status of the individual.

Although analyses of these data using wavelet transforms have yet to be done, differentiation between hyperthermia with and without dehydration, and dehydration alone using basic time domain and PSD analyses may be useful in generating algorithms to help determine physiological status. The WPSM is still under development and is envisioned to consists of an array of biosensors embedded in the clothing that would provide information to be used in casualty prevention and management as well as determining the individual's status. Successful application of the analyses derived from this animal model could be used to construct equivalent algorithms to predict physiological status under environmental extremes.

Acknowledgements

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, Department of Health and Human Services, revised 1996. The United States Army Research Institute of Environmental Medicine is an AAALAC-I accredited facility and will continue to adhere to the standards and requirements thereof. Citations of commercial organizations and trade names do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

References

Casadei, B., Cochrane, S., Johnston, J., et al., 1995. Pitfalls in the interpretation of spectral analysis of the heart rate variability during exercise in humans. Acta Physiologica Scandinavica 153, 125–131.

Cerutti, C., Gustin, M.P., Paultre, C.Z., et al., 1991. Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach. American Journal of Physiology 261, H1292–H1299.

Crandall, C.G., Zhang, R., Levine, B.D., 2000. Effects of whole body heating on dynamic baroreflex regulation of heart rate in humans. American Journal of Physiology 279, H2486–H2492.

Evans, J.M., Ziegler, M.G., Patwardhan, A.R., et al., 2001. Gender differences in autonomic cardiovascular regulation: spectral, hormonal, and hemodynamic indexes. Journal of Applied Physiology 91, 2611–2618.

Hainsworth, F.R., 1967. Saliva spreading activity and body temperature regulation in the rat. American Journal of Physiology 212, 1288–1291.

Hoyt, R.W., Riefman, J.R., Coster, T.W., Buller, M.J., 2002. Combat medical informatics: present and future. In: Kohane, I.S. (Eds.), Biomedical Informatics: One Discipline. Proceedings of the 2002 AMIA Annual Symposium, pp. 335–339.

Hubbard, R.W., Bowers, W.D., Matthew, W.T., et al., 1977. Rat model of acute heatstroke mortality. Journal of Applied Physiology 42, 809–816.

Hubbard, R.W., Matthew, C.B., Francesconi, R.P., 1982. Heat-stressed rat: effects of atropine, desalivation, or restraint. Journal of Applied Physiology 53, 1171–1174.

Kitney, R.I., 1975. Entrainment of the human RR interval by thermal stimuli. Journal of Physiology 252, P37–P38.

Kleiger, R.E., Stein, P.K., Bosner, M.S., Rottman, J.N., 1995. Time-domain measurements of heart rate variability. In: Malik, M., Camm, A.J. (Eds.), Heart Rate Variability. Futura Publishing Company, Inc., Armonk, NY, pp. 33–45.

Lieberman, H.R., Coffey, B.C., 2000. Preliminary finding from a new device for monitoring performance and environmental factors in the field. In: Friedl, K., Lieberman, H., Ryan, D.H., Bray, G.A. (Eds.), Pennington Center Nutrition Series: Countermeasures for Battlefield Stressors, Vol. 10. Louisiana State University Press, Baton Rouge, pp. 126–159.

Lipsitz, L.A., Mietus, J., Moody, G.B., Goldberger, A.L., 1990. Spectral characteristics of heart rate variability before and during postural tilt. Relations to aging and risk of syncope. Circulation 81, 1803–1810.

Marple, S.L., 1987. Digital Spectral Analysis. Prentice-Hall, Englewood Cliffs, NJ.

Matthew, C.B., 1997. Heat acclimation in telemetry equipped rat. Journal of Thermal Biology 22, 275–280.

Matthew, C.B., Hubbard, R.W., Francesconi, R.P., Szlyk, P.C., 1986. An atropinized heat-stressed rat model: dose response and pharmacokinetics. Aviation Space and Environmental Medicine 57, 659–663.

Nelskla, K., Yli-Hankala, A., Sjoberg, J., Korhonen, I., Kottila, K., 1999. Warming of insufflation gas during laproscopic hysterectomy: effect of body temperature and

the autonomic nervous system. Acta Anaesthesiologica Scandinavica 43, 974–978.

Perlini, S., Giangregorio, F., Coco, M., et al., 1995. Autonomic and ventilatory components of heart rate and blood pressure variability in freely behaving rats. American Journal of Physiology 269, H1729–H1734.

Pichot, V., Busso, T., Roche, F., et al., 2002. Autonomic adaptations to intensive and overload training periods: a laboratory study. Medicine and Science in Sports and Exercise 34, 1660–1666.

Pomeranz, B., MacAulay, R.J.B., Caudill, M.A., et al., 1985. Assessment of autonomic function in humans by heart rate spectral analysis. American Journal of Physiology 248, H151–H153.

Sayers, B.McA., 1973. Analysis of heart rate variability. Ergonomics 16, 17–32.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 93, 1043–1065.

Vatner, S.F., Pagani, M., 1976. Cardiovascular adjustments to exercise: hemodynamics and mechanisms. Progress in Cardiovascular Diseases 29, 91–188.